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# Comparison of immobilized poly-L-aspartic acid and poly-L-glutamic acid for chelation of metal cations

Lisa Malachowski, James A. Holcombe*

*Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712, USA*

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**Abstract**

Poly-L-aspartic acid (PLAsp) and poly-L-glutamic acid (PLGlu) were individually immobilized onto controlled pore glass (CPG) and compared according to their metal-binding capabilities in a solution of pH 7.0. The metal-binding capacities were calculated through the analysis of breakthrough curves generated by monitoring the metal concentrations on a flow injection–flame atomic absorption system. Capacities for individual metals were comparable and in the order of $Cu^{2+} \gg Pb^{2+} > Ni^{2+} \approx Cd^{2+} > Co^{2+} > Mn^{2+} \gg Na^{+}$. Elemental combustion analysis yielded polymer coverage on the CPG of approximately $4 \times 10^{12}$ to $5 \times 10^{12}$ chains/cm$^2$, when average chain lengths were used in the calculations. Formation constants and site capacities of both polymers for $Cd^{2+}$ were determined through equilibrium and breakthrough studies. The maximum log $K$ values for the strong sites were determined to be ~13 for both PLAsp and for PLGlu. Additionally, the metal selectivity of PLAsp and PLGlu was evaluated when breakthrough curves were run with several metals present in solution at one time. Both polymers showed selectivities in the order of their single metal-binding capacities, i.e., $Cu^{2+} > Pb^{2+} > Ni^{2+} \approx Cd^{2+}$. Both polymers exhibited similar binding trends and binding strengths for all of the metals studied. This likely reflects the absence of a predetermined tertiary structure of the polymers on the surface and the relatively high residue-per-metal ratio (~20:1), which places less stringent requirements on the steric hindrance between the side chains and the resultant "wrapping" of the peptide around the metal.


*Keywords:* Poly-L-aspartic acid; Poly-L-glutamic acid; Poly-L-aspartate; Poly-L-glutamate; Controlled pore glass; Ion exchange; Flow injection analysis; Flame atomic absorption spectrometry; Preconcentration; Remediation; Metal-binding peptide

## 1. Introduction

Recently, a significant amount of work has focused on the development of novel systems for remediation and preconcentration of heavy metals from contaminated waste streams. An interesting focus in this development has been the use of short chain, amino acid homopolymers immobilized onto a solid support [1–10]. By using amino acids as building blocks, with their various side chains, a variety of functionalities are available for coordination to metal ions. Through proper utilization of these various functionalities and the flexibility afforded by a surface-anchored linear polymer, the newly developed chelators can exhibit specificity and strong binding, highly desired characteristics in metal remediation. Additionally, the polypeptide has the inherent benefit of being non-toxic when discarded.

In previous studies, various polyamino acid chains have been immobilized onto controlled pore glass (CPG) [1,3,5,7,11,12], gold minigrids [4], porous carbon [10], and both silica- and cellulose-based membranes [2,6,8,9] and their metal-binding abilities were characterized. In all instances the immobilized polyamino acid chains were proven to be effective metal chelators.

Studies involving immobilized poly-L-cysteine (PLCys), with its thiol side chain functionality, demonstrated its preference for soft acid metals such as $Cd^{2+}$ and $Pb^{2+}$ over metals such as $Co^{2+}$ and $Ni^{2+}$ [1,3,8,10]. In contrast to PLCys, immobilized poly-L-aspartic acid (PLAsp) [4–7,9] and poly-L-glutamic acid (PLGlu) [2,6,8,9] with carboxylate side chains, showed significant $Cu^{2+}$ and $Pb^{2+}$ capacity. It has also been shown that poly-L-histidine (PLHis), with its imidazole side chain, is capable of binding metal cations at neutral pH values and metal oxyanions (e.g., the chromates, arsenates, and selenites) at acidic pH values, where the imidazole is protonated [12]. The general binding trends in all

* Corresponding author. Tel.: +1 512 4715140; fax: +1 512 4710985.
*E-mail address:* holcombe@mail.utexas.edu (J.A. Holcombe).

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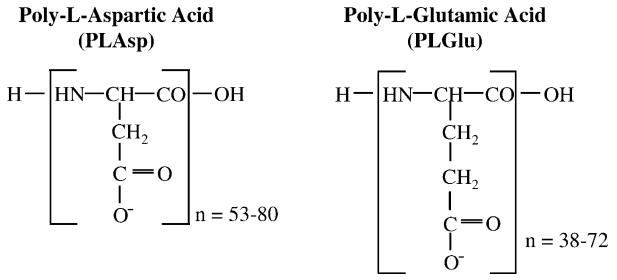


Fig. 1. Structures of poly-L-aspartic acid and poly-L-glutamic acid.

of the above cases follow expected general trends based on the available cationic or anionic functionalities on the peptide.

However, it is not obvious whether more subtle changes impact metal chelation. In this paper, PLAsp and PLGlu (Fig. 1) were evaluated. The primary difference between the two amino acids is the added methylene group in the side chain of glutamic acid. The purpose of this study was to compare and evaluate the metal-binding characteristics of PLAsp and PLGlu immobilized onto CPG in an attempt to determine if this slight difference in the two metal-binding functional side chains has an effect on the binding strengths (e.g., formation constants) or on the number of metals binding to these polymers.

## 2. Experimental

### 2.1. Instrumentation

A Perkin-Elmer model 4000 flame atomic absorption (FAA) spectrophotometer with an acetylene/air flame was used for all metal determinations. Hollow cathode lamps for the metals of interest were operated at the currents recommended by their manufacturers. Wavelengths for Cd, Co, Cu, Mn, Na, Ni, and Pb were 228.8, 240.7, 324.8, 279.5, 589.0, 232.0, and 283.3 nm, respectively. A monochromator bandpass of 0.2 nm was used for Co, Mn, and Ni; 0.4 nm for Na; and 0.7 nm for Cd, Cu, and Pb.

A simple flow injection manifold consisting of an 8-roller peristaltic pump (Ismatec minicartridge MS-REGLO) and two 2-way, double inlet rotary valves (Rheodyne 5020) was used. All connections were made with 0.76 mm i.d. PTFE tubing [5].

Approximately 0.1 g of immobilized PLAsp-CPG and immobilized PLGlu-CPG were each packed into two separate 3 mm i.d. × 25 mm long glass columns with 70 mm PTFE frits (Omnifit). The empty bed volume of the column, packed with controlled pore glass is approximately 0.085 mL. A Kel-F tee was placed between the column and the nebulizer to provide air compensation and to minimize noise.

A Varian Ultramass inductively coupled argon-plasma mass spectrometry (ICP-MS) system was used for analysis of breakthrough curves in the determination of selected conditional formation constants.

### 2.2. Reagents

All chemicals were reagent grade unless noted; and deionized, distilled water was used to prepare solutions. All glasswares were soaked in 4 M $HNO_3$ overnight before use. Poly-L-aspartic acid (Sigma) [DP(vis) 80, MW(vis) 11,000] and poly-L-glutamic acid (Sigma) [DP(vis) 72, MW(vis) 10,900] were used as received. The controlled pore glass (Sigma, PG240-120) had a mean pore diameter of 22.6 nm and a mesh size of 80–120. Other reagents included 3-aminopropyltriethoxysilane (98%), nitric acid (Aldrich); acetic acid (Fisher Scientific); ammonium acetate, ammonium hydroxide (Mallinckrodt); gluteraldehyde (25%) (Sigma); and ethylenediaminetetraacetic acid (EDTA) (EM Science). Stock solutions of $Cd^{2+}$ (Inorganic Ventures); $Cu^{2+}$ and $Pb^{2+}$ (SCP Science) atomic absorption standards were used to prepare the 10 ppm loading solutions for the metal-binding experiments. For $Co^{2+}$, $Na^{+}$, $Ni^{2+}$ (Baker), $Mn^{2+}$ (Matheson, Coleman & Bell) the loading solutions were prepared from standardized solutions of the reagent grade nitrate salt. A 0.5 M ammonium acetate stock solution was prepared and purified using a 100–200 mesh Chelex 100 (Bio-Rad) ion exchange column.

### 2.3. Immobilization of PLAsp and PLGlu onto CPG

The preparation of the PLAsp-CPG and the PLGlu-CPG followed the procedure that has previously been described in the immobilization of PLHis [12]. The process was modified slightly by attaching the polyamino acid in a solution of pH 8.0. This elevated pH should enhance the efficiency of this linkage.

Elemental combustion analysis for carbon was conducted by MHW Labs (Phoenix, AZ, USA), in duplicate on the functionalized glass at various stages of the immobilization to estimate the surface coverage of the polymers on the glass.

### 2.4. Metal-binding characteristics of PLAsp-CPG and PLGlu-CPG

The previously described flow injection analysis system was utilized in all metal-binding experiments. The pumps, tubing, hollow cathode lamp, and flame were warmed up for at least 15 min prior to use. After conditioning the column, unretained, acidified (pH < 1.0) metal standards were run through the column and FAA system. The absorbance values were used to prepare calibration curves.

Upon completion of the calibration curves, a 0.05 M ammonium acetate solution (pH 7.0) was pumped through the column for 2 min at 1 mL/min to recondition the column to the neutral pH and solution conditions. The metal-binding solutions were prepared by dilution from the metal standards into 0.05 M ammonium acetate, diluted from the stock, and adjusted to pH 7.0 by dropwise addition of acetic acid or ammonium hydroxide. The 10 ppm ammonium acetate–metal solution was then introduced onto the column at a flow rate

of 1 mL/min and the effluent concentration was detected by FAA to ultimately produce a breakthrough curve. Once the effluent concentration equaled the influent concentration, the sample flow was stopped. Ammonium acetate was passed through the column and emptied into waste for ∼10 s (i.e., 0.16 mL) to remove the remaining metal-containing solution from the lines and the column dead volume. The metals were stripped from the column by flowing 0.1 M $HNO_3$ for 5 min at 1 mL/min through the column and collecting the effluent in a 25 mL volumetric flask for subsequent analysis by FAA. Although it has been shown in previous studies that the metals are stripped from the column in only a few hundred microliters of acid [5], 5 mL ensured complete removal as well as provided sufficient volume at a reasonable concentration to permit FAA analysis of the solution using the conventional nebulizer. The strip solution was not analyzed by direct transfer from the column to the FAA because the relatively rapid release of metals from the column produced a solution plug whose metal concentration greatly exceeded the dynamic range of the detection system. Breakthrough curves and strip solution data were analyzed for each of the target cations ($Cd^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Mn^{2+}$, $Na^{+}$, $Ni^{2+}$, and $Pb^{2+}$), resulting in a relative binding capacity for each of the metals. This procedure was followed on both the PLAsp-CPG column and the PLGlu-CPG column. All metal-binding experiments were performed in triplicate on both columns.

In selected studies, where the ICP-MS was employed because of its lower limits of detection, the same procedure described above was employed.

### 2.5. Evaluation of stability constants

Stability constants on both PLAsp and PLGlu were determined using $Cd^{2+}$. The on-column method of determining stability constants has been previously described [1,3]. Determination of $K_f$ of the stronger sites was attempted by equilibrating the PLAsp-CPG or the PLGlu-CPG with known excess concentrations of EDTA and $Cd^{2+}$, which lowered the free $Cd^{2+}$ concentration and should permit evaluations without generating concerns about the loss of $Cd^{2+}$ to the container walls because of the metal buffer provided by the Cd–EDTA system. The stability constants for $Cd^{2+}$ and EDTA are well documented, and binding characteristics of $Cd^{2+}$ for PLAsp and PLGlu are experimentally measured in this study. In brief, an analytical solution of a fixed volume (25 mL), containing a known concentration of standardized EDTA and a known concentration of $Cd^{2+}$ in 0.05 M ammonium acetate at pH 7.0 was continuously recirculated through the columns for 18 h in order to ensure establishment of equilibrium of mobile and bound phases. The concentration of the metal remaining in the solution and the amount of metal that was bound to the column were then determined, which permitted determination of bound and free concentrations. From knowledge of the equilibrium constant of the Cd–EDTA complex and the initial and final $Cd^{2+}$ concentration in solution, the formation constant of the metal–peptide complex and the number of sites were determined. In addition, formation constants were also estimated by analyzing the early time baseline region of $Cd^{2+}$ breakthrough curves in the absence of EDTA on both polymers using ICP-MS. The improved sensitivity of the ICP-MS permits detection of the small amounts of $Cd^{2+}$ exiting the column even in the initial stages of flow when strong binding sites would still be available.

### 2.6. Mixed metal solution binding studies

Breakthrough analysis was conducted in the same manner outlined above, except that a single solution containing 10 ppm each of $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Pb^{2+}$ in 0.05 M ammonium acetate (pH 7.0) was passed through the column and the concentration of each of the metals in the effluent was monitored sequentially using FAA spectrophotometer. Strip solutions were also analyzed by FAA spectrophotometer for verification of bound metal. This procedure was followed on both the PLAsp-CPG column and the PLGlu-CPG column and all metal-binding experiments were performed in duplicate on both columns.

## 3. Results and discussion

### 3.1. Elemental analysis

Elemental combustion analysis yielded a %C of 3.14 ± 0.21 for the precursor gluteraldehyde functionalized CPG, 1.84 ± 0.26 for the PLAsp-CPG, and 2.33 ± 0.26 for the PLGlu-CPG. This corresponds to a surface coverage of 1.40 (±0.10) × $10^{14}$ gluteraldehyde residues/$cm^2$ of CPG, assuming a surface area of 94 $m^2$/g of CPG (Sigma). Due to the variability and uncertainty associated with the chain length of the PLAsp and PLGlu, no speculation was made as to the polymer coverage and results will be reported only as amino acid residues/g of support. Using the %C obtained from the elemental combustion analysis, the PLAsp coverage was determined to be 2.45 (±0.35) × $10^{14}$ aspartic acid residues/$cm^2$ of CPG and the PLGlu coverage was determined to be 2.49 (±0.28) × $10^{14}$ glutamic acid residues/$cm^2$ of CPG. The statistically indistinguishable coverages of the two polymers to the CPG allow a direct comparison of metal capacities to be made between these two systems without correcting for differences in coverage.

### 3.2. Metal-binding characteristics of PLAsp-CPG and PLGlu-CPG

The PLAsp-CPG and PLGlu-CPG column capacities and breakthrough curves were obtained for $Cd^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Mn^{2+}$, $Na^{+}$, $Ni^{2+}$, and $Pb^{2+}$ using separate 10 ppm influent solutions of the metal in 0.05 M ammonium acetate at pH 7.0. The influent was pumped through the column at

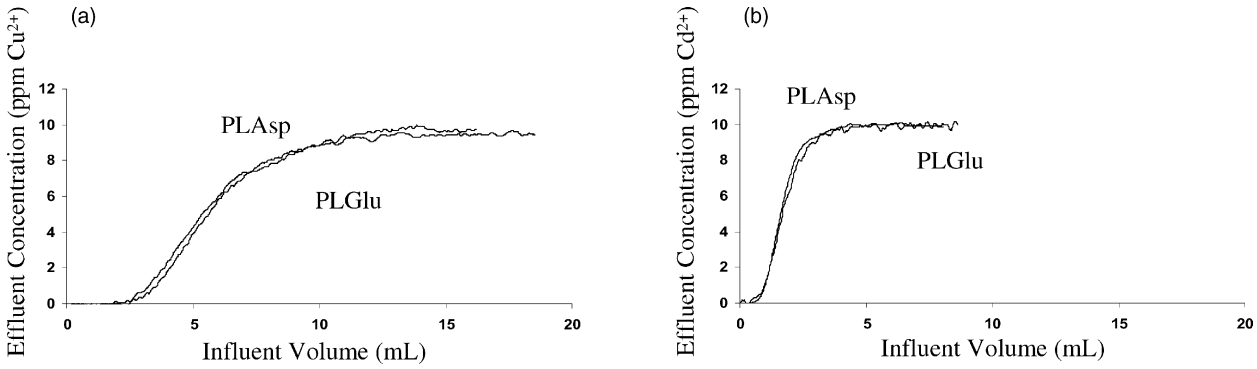


Fig. 2. Breakthrough curves on PLAsp-CPG and PLGlu-CPG for (a) $Cu^{2+}$ and (b) $Cd^{2+}$. All solutions were 10 ppm in the respective metal and 0.05 M ammonium acetate; pH 7.0.

1.0 mL/min and the metal concentration in the effluent was measured by FIAS-FAA as a function of influent volume. Representative breakthrough curves are shown in Fig. 2. The early-time, flat baseline region present on the curves is indicative of strong metal-binding. The sloped regions represent the weaker metal-binding sites. By integrating the breakthrough curve, the total amount of metal retained on the column can be determined. These values are validated using the results from the stripped solutions. Since the breakthrough and strip data were in good agreement, the capacities calculated from breakthrough data and from strip data were averaged [5,7]. Table 1 contains a summary of the metal-binding results for PLAsp-CPG and PLGlu-CPG. As seen in these data, PLAsp-CPG and PLGlu-CPG have similar capacities for all the metals evaluated and follow a similar metal-binding trend ($Cu^{2+} \gg Pb^{2+} > Ni^{2+} \approx Cd^{2+} > Co^{2+} > Mn^{2+} \gg Na^{+}$) as that reported by Gulumian et al. [13] for negative oxygen donors, such as carboxylates, in biological systems. It is also in good agreement with previous work on immobilized PLAsp [5,7], and on glutamic acid-bonded silica [14].

It has been shown previously that residual functionalities from each step of the immobilization procedure do not contribute significantly to the metal-binding capacity of these systems [5,12]. Therefore, this study is a direct comparison of PLAsp and PLGlu because the immobilized polymer is the main component of metal-binding.

Table 1
Metal-binding capacities on PLAsp-CPG and PLGlu-CPG columns determined from breakthrough curves and strip data

| Metal ion | Capacity (μmol/g CPG) on PLAsp-CPG | Capacity (μmol/g CPG) on PLGlu-CPG |
|---|---|---|
| $Cu^{2+}$ | 14.1 ± 1.3 | 13.9 ± 1.1 |
| $Pb^{2+}$ | 6.1 ± 0.2 | 6.8 ± 0.2 |
| $Ni^{2+}$ | 2.6 ± 0.2 | 2.7 ± 0.4 |
| $Cd^{2+}$ | 2.2 ± 0.3 | 2.7 ± 0.3 |
| $Co^{2+}$ | 1.91 ± 0.05 | 1.99 ± 0.06 |
| $Mn^{2+}$ | 1.7 ± 0.1 | 1.59 ± 0.01 |
| $Na^{+}$ | 0.34 ± 0.09 | 0.39 ± 0.08 |

pH 7.0, flow rate = 1 mL/min of 10 ppm influent solutions, triplicate measurements. Uncertainties expressed as sample standard deviations, reflect measurement uncertainties only.

Studies using NMR and polarography have been conducted to elucidate the specific metal–ligand interaction of these polymers [15–18]. In each of these studies the main metal-binding functionality is shown to be the carboxylate side chain. As a result, all of the current metal-binding studies were conducted at pH 7.0, which is significantly above the $pK_a$ values for the carboxylate side chain on PLAsp and PLGlu, which are reported to be approximately 5.4 and 4.4, respectively [2,5,14,16,19,20]. With pH $\gg$ $pK_a$ maximum binding could occur since the side chain carboxylates are completely deprotonated and the chains are fully extended [7].

Structural determination has shown that PLGlu undergoes a helix-to-coil transition as the pH increases, most likely due to the deprotonation of the carboxylic acid groups [19–29]. Studies of this helix-to-coil conformational change have also considered the effects of polymer concentration [30], time [31], size of cations in solution [32], salt concentration and ionic strength [23,33–35], and the presence of specific cations in solution [15,18,36–48]. Most of the studies conducted in the presence of various metals agree that the metal ions have a significant effect on the conformation of PLGlu. More specifically, the metal–PLGlu interaction shifts the helix-to-coil transition to a higher pH.

Similarly, poly(α-L-Asp) also undergoes a helix-to-coil transition [49–51]. It also exists as a compact helix at low pH values but is present as an extended random coil at higher pH values. The conformation change occurs in both of these polymers because at low pH values the H-bonds between the amide and carboxylate groups of the biopolymer stabilize the helix and as the pH increases the carboxylates become charged, resulting in a strong electrostatic repulsion that destabilizes the helix [21,52]. Although, it has been shown that in some instances the helix forming ability of PLAsp may be less that of PLGlu [26], Saudek and coworkers [49–51] suggest that this is due to a low helix forming ability of the β form of PLAsp. It has also been suggested that the lack of evidence of a helical form of PLAsp is due to

PLAsp forming a helix at a lower degree of ionization where it also precipitates very quickly [50]. This may also be the due to the fact that the carboxylate side chains of PLAsp are shorter than those of PLGlu, resulting in a stronger electrostatic repulsion between the ionized carboxylates [50,53]. In other words, it takes a lesser degree of ionization in PLAsp than in PLGlu to cause the same amount of repulsion. Additionally, it has been shown that due to a decrease in the hydrophobic interactions, which stabilizes the helix, the helix forming ability of certain polymers decreases with a decrease in the number of $CH_2$ groups in the side chain [50]. In short, these studies show that PLGlu and PLAsp undergo similar, but not identical, conformational changes.

In addition to determining absolute capacities of the polymer functionalized CPG for various metals, these metal-binding studies provide information regarding the metal-binding efficiency of the polymer chains when coupled with the polymer coverage data, calculated from the %C determined by elemental combustion analysis. The manufacturer (Sigma) determined the degree of polymerization of PLAsp by viscosity to be ∼80 and by multiangle laser light scattering (MALLS) to be ∼53. For PLGlu the degree of polymerization determined by viscosity is ∼72 and by low angle laser light scattering (LALLS) it is ∼38. This range in the degree of polymerization for both polymers was used in the error analysis and, when needed, propagated as the standard deviation of the mean. Based on a degree of polymerization of PLAsp of 53–80 residues (Sigma) and a $Cu^{2+}$ capacity of 14.1 ± 1.3 μmol $Cu^{2+}$/g CPG for PLAsp-CPG, the number of $Cu^{2+}$ atoms bound per chain was determined to be 2.4 ± 0.6 $Cu^{2+}$/chain (or 27.2 ± 2.5 aspartic acid residues/$Cu^{2+}$ bound). The degree of polymerization for PLGlu is 38–72 residues (Sigma), and with a $Cu^{2+}$ capacity of 13.9 ± 1.1 μmol $Cu^{2+}$/g CPG, PLGlu-CPG binds 2.0 ± 0.7 $Cu^{2+}$/chain (or 28.0 ± 2.2 glutamic acid residues/$Cu^{2+}$ bound). It is obvious from the large residue-to-metal ratios that the polymers have a large number of unused carboxylate binding functionalities that are probably not involved in coordinating the metal cations. Since it is reasonable to assume that the polymers wrap around the metal to achieve the necessary tertiary structure to bind the metal [54]; the binding similarities between PLAsp and PLGlu suggest that any differences in the chain flexibility, p$K_a$ values or steric hindrance is negligible in this application.

### 3.3. Evaluation of conditional stability constants

As mentioned previously, the flat baseline region present on the breakthrough curves seen in Fig. 2 is indicative of strong metal-binding and the sloped regions represent the weaker metal-binding sites. Binding studies using EDTA in solution to limit the free $Cd^{2+}$ concentration were used for the determination and calculation of the free $Cd^{2+}$ in solution and the amount of metal bound to the PLAsp and the PLGlu. Formation constants for $Cd^{2+}$ binding to PLAsp-CPG and PLGlu-CPG were estimated using the relationship in Eq. (1).

$$CdL + X \rightleftharpoons CdX + L \quad (1)$$

where X is PLAsp or PLGlu and L is EDTA ($Y^{4-}$). The conditional stability constant for $CdY^{2-}$ ($1.15 \times 10^{13}$) was based on a literature value [55].

Previous studies have used Scatchard analysis to estimate conditional stability constants for metal-binding by poly-L-cysteine [3,10]. The Scatchard function is used to determine conditional stability constants by the following expression:

$$\frac{[CdX_i]}{[Cd]} = K_i(n_i - [CdX_i]) \quad (2)$$

where $[CdX_i]$ (as μmol/g resin) is the number of complexed sites of type $i$, $n_i$ is the total concentration (as μmol/g resin) of type $i$ sites, and $K_i$ is the stability constant for the $i$th site.

However, it has previously been shown that PLAsp functionalized silica is capable of binding both cations and anions [56], and this analysis fails to consider the possibility that a negatively charged $CdY^{2-}$ complex may bind to residual amine functionalities on the surface that remain after immobilization of the polymer chains to the CPG. If the $CdY^{2-}$ complex binds to the support, the *apparent amount* of bound $Cd^{2+}$ will be higher since some of the complexed Cd is present as the $CdY^{2-}$ species. As a result, the calculated formation constant of PLAsp and PLGlu to $Cd^{2+}$ in the presence of EDTA is artificially high and the resulting calculated value for $K$ can be considered the upper limit.

In order to determine the range of the actual formation constant, a minimum value of $K$ was determined through analysis of the baseline region of the $Cd^{2+}$ (*without EDTA*) breakthrough curves using ICP-MS. The detection limit of the ICP-MS for $Cd^{2+}$ was determined to be 0.66 ppb. Since Cd was still not detected during the flat baseline region of the breakthrough curve using the ICP-MS, the free $Cd^{2+}$ in the solution exiting the column must be less than 0.66 ppb. Substituting this information into the equilibrium expression results in the determination of $K \geq 2.0 \times 10^8$ for both polymers. Thus, the calculated stability constants and site capacities for PLAsp and PLGlu are $2.0 \times 10^8 < K < 3.8\ (\pm 0.5) \times 10^{13}$, $n = 0.22\ (\pm 0.04)$ μmol/g and $2.0 \times 10^8 < K < 3.2\ (\pm 1.0) \times 10^{13}$, $n = 0.19\ (\pm 0.08)$ μmol/g, respectively. These results indicate that PLAsp and PLGlu may bind similar amounts of the Cd–EDTA complex and have the same number of sites and therefore bind equal amounts of free $Cd^{2+}$. This demonstrates that both polymers have similar binding capabilities for $Cd^{2+}$.

It is interesting to note that Lumb and Martell [57] potentiometrically determined the chelate stability constants ($\log K$) for Cd–Asp and Cd–Glu to be 4.39 ($K = 25{,}000$) and 3.9 ($K = 7900$), respectively, for the homogeneous solution containing the metal and amino acid monomers. With the free amino acids in solution there exists an additional carboxylate functionality at one terminus for each residue

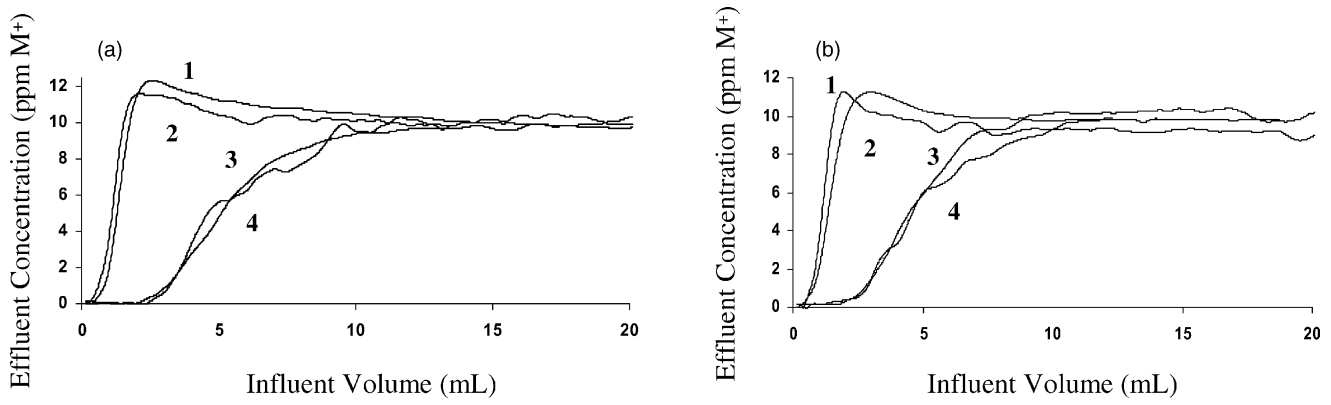


Fig. 3. Breakthrough curves on (a) PLAsp-CPG and (b) PLGlu-CPG from a multi-metal solution containing 10 ppm each of (1) $Cd^{2+}$, (2) $Cu^{2+}$, (3) $Ni^{2+}$, and (4) $Pb^{2+}$ in 0.05 M ammonium acetate; pH 7.0.

and an available amine functionality at the other terminus. In fact, they suggest a metal-binding complex that utilizes both the carboxylate and amine termini on each amino acid residue.

### 3.4. Mixed metal solution binding studies

To determine how the column capacity for a specific metal is affected by the presence of other metals, a multi-metal solution was passed through the column; and breakthrough and strip analyses were conducted. The solution that was passed through the column contained 10 ppm each of $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Pb^{2+}$ in pH 7.0, 0.05 M ammonium acetate. Fig. 3 contains an example of the multi-metal breakthrough curves on PLAsp-CPG and PLGlu-CPG. The calculated capacities can be seen in Table 2. Once again, these values represent flow data and true equilibrium has likely not been achieved. As the data indicate, the binding selectivity is consistent with that determined through single metal studies and also with that previously published for carboxylate ligands [13]. The capacities were in the order $Cu^{2+}$ > $Pb^{2+}$ > $Ni^{2+}$ > $Cd^{2+}$. While the calculated capacities for each metal in the multi-metal solution were lower than the capacities determined for each metal individually, the total metal-binding capacity using the mixed metals solution is greater than the capacity for $Cu^{2+}$ when run alone. ($Cu^{2+}$ is cited since it showed the greatest individual capacity of the metals tested.) Specifically, the total amount of metal bound in the mixed metal solution is approximately 20% larger than that found for the solution containing only $Cu^{2+}$. This is consistent with the idea that these polymers have no predetermined binding "cavity", unlike nature's metal-binding proteins or traditional metal chelators, such as EDTA or crown ethers. These relatively short homopolymers appear capable of adopting a different tertiary structure in the presence of various metals, or a multi-metal solution. It may even be suggested that the formation of one metal-binding cavity may instill cooperative binding to improve the likelihood of another metal-binding through the new tertiary structure by altering the proximity of adjacent ligands in a favorable fashion for additional metals to bind. It is also important to note the maxima of ~12 ppm in the $Cd^{2+}$ and $Ni^{2+}$ curves is higher than the influent concentration of 10 ppm (Fig. 3). Initially, when all of the sites are free, all of the metals begin to bind. Due to the selectivity sequence of carboxylate ligands, as the free sites become limited the $Cu^{2+}$ and $Pb^{2+}$ are able to displace the $Cd^{2+}$ and $Ni^{2+}$. This supports the idea that the strong binding sites for $Cu^{2+}$ and $Pb^{2+}$ are stronger than the strong sites for $Cd^{2+}$ and $Ni^{2+}$. Hence, the peaks in Fig. 3 for $Cd^{2+}$ and $Ni^{2+}$ at approximately 3 mL of influent volume. Once $Cd^{2+}$ and $Ni^{2+}$ are displaced by $Cu^{2+}$ and $Pb^{2+}$, their concentrations level off at 10 ppm, i.e., no additional net binding of these metals. Ritchie et al. have previously reported similar results on PLGlu with $Cd^{2+}$ and $Pb^{2+}$ [8].

Table 2
Multi-metal solution binding capacities on PLAsp-CPG and PLGlu-CPG columns

| Metal ion | Capacity (μmol/g CPG) on PLAsp-CPG | Capacity (μmol/g CPG) on PLGlu-CPG |
|---|---|---|
| $Cu^{2+}$ | 13.0 ± 1.2 | 11.8 ± 2.1 |
| $Pb^{2+}$ | 4.0 ± 0.3 | 4.3 ± 0.8 |
| $Ni^{2+}$ | 1.2 ± 0.1 | 0.9 ± 0.1 |
| $Cd^{2+}$ | 0.5 ± 0.1 | 0.75 ± 0.04 |

10 ppm $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Pb^{2+}$ in 0.05 M ammonium acetate influent, pH 7.0, flow rate = 1 mL/min, triplicate measurements.

## 4. Conclusions

This study showed that PLAsp-CPG and PLGlu-CPG had very similar metal-binding characteristics despite the extra methyl group in the carboxylate side chain of PLGlu. These polymers are unlike nature's metal-binding proteins that have a predetermined tertiary structure for metal-binding. As a result, in a protein, the substitution of an Asp for a Glu, or vice versa, may cause a significant change in the metal-binding capabilities as might be suggested, for example, by the results of Jablonski and Morrow [58].

Metal-binding experiments on PLAsp-CPG and PLGlu-CPG demonstrated that both polymers are effective metal cation chelators and exhibit similar binding selectivity for the metals tested. Both polymers possess a metal-binding trend of $Cu^{2+} \gg Pb^{2+} > Ni^{2+} \approx Cd^{2+} > Co^{2+} > Mn^{2+} \gg Na^{+}$, with a maximum capacity for $Cu^{2+}$ at $\sim$14 μmol/g CPG. Mixed metal studies clearly demonstrated the improved selectivity of $Cu^{2+}$ and $Pb^{2+}$ over $Cd^{2+}$ and $Ni^{2+}$ for both polymers by showing that $Cu^{2+}$ and $Pb^{2+}$ were able to displace bound $Cd^{2+}$ and $Ni^{2+}$ from the columns.

In general, these polymers behaved very similarly in all of the studies conducted, and should be equally suitable for trace metal preconcentration and remediation from natural and industrial waste streams.

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